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Creation of the Fontan circulation in sheep: a survival model[†]

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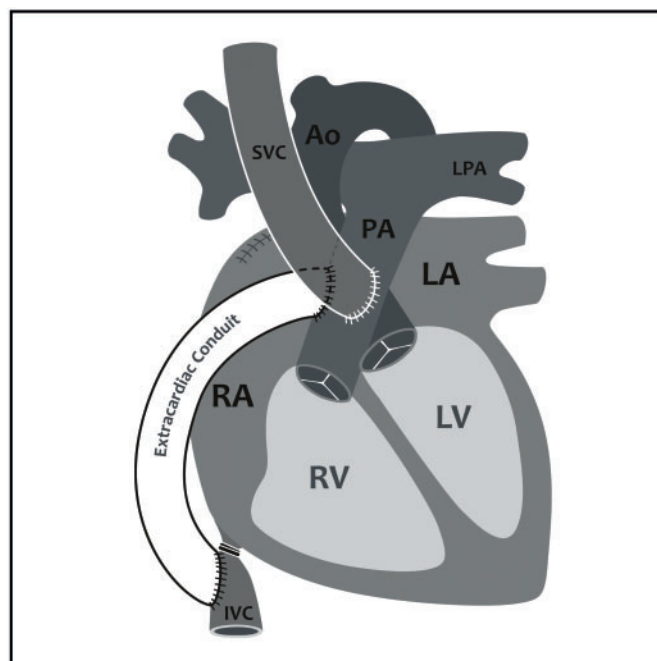
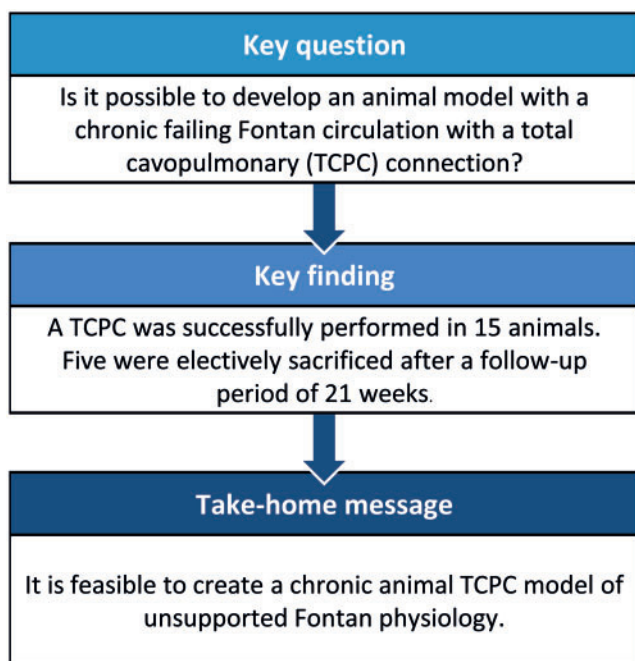
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Abstract

OBJECTIVES: Patients with a single ventricle survive thanks to the Fontan palliation. Nevertheless, there is a growing number of Fontan patients with progressive heart failure. To validate therapeutic options in these patients, we developed a chronic Fontan large animal model.

METHODS: A Fontan circulation was surgically created in 15 sheep. The superior vena cava was anastomosed end-to-side to the pulmonary artery. The inferior vena cava was connected to the pulmonary artery by an ePTFE conduit, and the inferior vena cava-right atrium junction was ligated.

RESULTS: Total cavopulmonary connection was successfully performed in all 15 animals. After creation of the Fontan circulation, central venous pressure increased from 4 [interquartile range (IQR) 3–6] mmHg to 16 (IQR 14–17) mmHg, mean arterial blood pressure decreased from 68 (IQR 54–75) mmHg to 52 (IQR 50–61) mmHg and cardiac output decreased from 5.1 (IQR 4.6–6.8) l/min to 1.7 (IQR 1.3–2.7) l/min. Five animals were electively sacrificed after a follow-up period of 21 weeks.

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CONCLUSIONS: These results demonstrate that it is feasible to create a chronic animal model with unsupported Fontan circulation. This animal model not only opens perspectives to investigate the pathophysiology of the failing Fontan circulation, but also provides the possibility to study therapeutic options such as the effect of mechanical circulatory support in the failing Fontan physiology.

Keywords: Total cavopulmonary connection • Failing Fontan circulation • Congenital heart disease • Experimental study • Fontan procedure

INTRODUCTION

The outcome for patients with a functional single ventricle has improved greatly since the Fontan circulation was first described in 1971 [1]. Over the past decades, many modifications to the original technique have been introduced, of which the total cavopulmonary connection (TCPC) is the most prevailing [2]. In this staged procedure, the systemic and pulmonary circulations are separated and the systemic venous return is directly connected to the pulmonary arteries. As a result, the arterial oxygen saturation is normalized and the single ventricle is no longer subjected to volume overload. However, as a cost, the systemic venous pressure is notably elevated and cardiac output (CO) is decreased, both at rest and during exercise [3]. These and other factors can lead to progressive failure of the Fontan circulation. It is estimated that about 30% of all Fontan patients will develop a failing Fontan circulation [2].

Heart transplantation will be the final option for many of these patients. However, the failing Fontan physiology and a history of multiple previous interventions make them less than ideal candidates for transplantation. Even after a successful transplantation, the reported 10-year survival of these patients is only 60% [4]. Ventricular assist devices have been successfully used to support the failing left ventricle (LV) in chronic heart failure conditions, [5, 6] but they are not applicable to the failing Fontan circulation. As failing Fontan patients lack 1 ventricle, the requirements for such a mechanical support system differ from those for conventional ventricular assist devices. In recent years, there is an increasing interest in developing mechanical support systems specific for the failing Fontan configuration [7–12]. Therefore, there has also

been increasing interest in models that test the capability of these devices to support the Fontan circulation *in vivo*. These experimental studies all emphasized that the elevated central venous pressure resulting from an acute exclusion of the right ventricle (RV) is not capable of maintaining an adequate CO. This results in a high incidence of ventricular dysfunction, leading to a serious impediment to survival of the animals. This limits these studies to acute experiments or models of an incomplete bypass of the right heart [13–23].

There is a growing population of failing Fontan patients that will require innovative and creative technological advancements that are capable to support their Fontan circulation. A chronic failing Fontan animal model with a total cavopulmonary connection could facilitate future studies on the pathophysiology of the failing Fontan circulation. A better understanding of the pathophysiological processes behind Fontan failure might give us the opportunity to adopt early intervention strategies and prevent late failure in these failing Fontan patients.

It was the goal of this study to develop a chronic failing Fontan animal model by establishing a TCPC.

MATERIALS AND METHODS

Animals

This study was performed with the approval of and adherence to the guidelines of the animal ethics committee of the KU Leuven, according to the 'Principles of Laboratory Animal Care', formulated by the National Society for Medical Research, and in



Figure 1: Schematic overview of the protocol. The animals were trained on a treadmill for 2 weeks prior to the procedure. Two days before the procedure, an exercise test was performed. Subsequently, the TCPC procedure was performed. After recovery of the surgery (usually 2–4 weeks post-TCPC), the exercise test was repeated. During the post-TCPC follow-up period, the animals received diuretics daily, and once per week their weight and central venous pressure were recorded. CVP: central venous pressure; TCPC: total cavopulmonary connection.

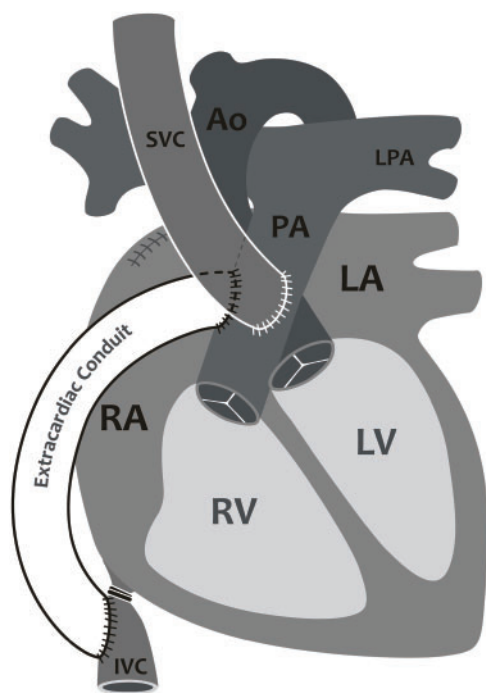


Figure 2: Schematic illustration of the experimental technique. Ao: Aorta; IVC: inferior vena cava; LA: left atrium; LPA: left pulmonary artery; LV: left ventricle; PA: main pulmonary artery; RA: right atrium; RV: right ventricle; SVC: superior vena cava.

compliance with the European Commission Directive 2010/63/EU. Fifteen adult female sheep (Swifter × Charollais crossbreed), median weight 63.4 [interquartile range (IQR) 62.8–68.5] kg, were obtained from the Zoötechnical Center of the KU Leuven.

Protocol

By way of an overview of the protocol (Fig. 1), a Fontan circulation was induced in all animals by establishing a TCPC with an extracardiac conduit (Figs 2 and 3). The procedure was performed without the use of cardiopulmonary bypass or blood transfusions. Preoperative and postoperative haemodynamics were recorded in all animals. The animals received diuretics daily, but no anticoagulating or platelet-inhibiting agents were administered. A subgroup of animals ($n=8$) were trained for 2 weeks to walk (1–2 km/h) on a motor-driven treadmill (Fit fur life treadmills, Fernhurst, UK) and performed an exercise test 2 days before the procedure. After recovery from surgery, this exercise test was repeated ($n=4$).

Surgical procedures

After sedation with ketamine (4 mg/kg intravenously), anaesthesia was induced (5% in oxygen, via face mask) and maintained (1–3%) with isoflurane. An endotracheal tube was inserted for mechanical ventilation (13–15/min; tidal volume 0.7 times body weight). An orogastric tube was placed to prevent ruminal distension. An arterial pressure line was placed in an ear artery for invasive arterial pressure monitoring. A triple lumen deep venous catheter was placed in the right jugular vein for fluid administration and central venous pressure monitoring. A maintenance

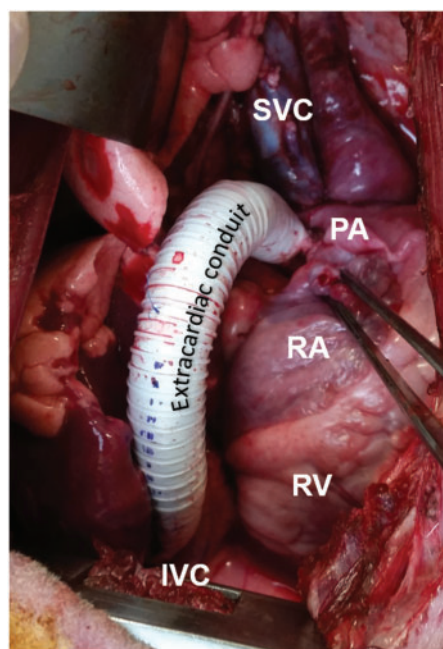


Figure 3: Intraoperative picture of the total cavopulmonary connection. IVC: inferior vena cava; PA: main pulmonary artery; RA: right atrium; RV: right ventricle; SVC: superior vena cava.

intravenous drip (0.9% NaCl; 20 ml/h) was started, and gentamicin (6.6 mg/kg) and penicillin (40 000 IU/kg) were administered intravenously. The animal was placed on the operating table in a right lateral recumbent position, surgically scrubbed and draped to expose the right hemithorax.

A right lateral thoracotomy was performed in the 3rd intercostal space. The ribs were retracted to provide adequate exposure of both caval veins. The pericardium was incised anterior of the phrenic nerve to expose the right atrium and the pulmonary artery. The superior caval vein and the pulmonary artery were freed from the surrounding tissue, and purse string sutures were placed on the superior vena cava and the right atrium. Heparin (200 U/kg) was administered. The superior vena cava and the right atrium were each cannulated with a venous 18-Fr cannula (Edwards Lifesciences, Irvine, CA, USA), and the cannulas were connected to create a superior vena cava–right atrium bypass. The superior vena cava was cross-clamped proximally and distally and transected. The proximal section was closed with a continuous running suture (polypropylene 4/0). A partial occluding clamp was placed on the cranial side of the pulmonary artery, and the superior vena cava was anastomosed end-to-side to the pulmonary artery. The clamps were opened and the cannulas were removed. A partial occluding clamp was placed on the inferior vena cava, and a valveless ePTFE graft (18–22 mm OD) was anastomosed to the inferior vena cava. The right lateral side of the pulmonary artery was partially clamped, and the graft was connected end-to-side to the pulmonary artery. The anastomoses were all performed using a polypropylene 5/0 continuous running suture. The graft was de-aired and all the clamps were removed. The connection between the inferior vena cava and the right atrium was ligated permanently (polyethylene terephthalate 2/0). Careful haemostasis was performed and a chest drain was placed in the right pleural space before the chest was

closed. Animals were weaned from ventilation as soon as there was spontaneous respiration. Per-operative hypotension was treated with an intravenous infusion of phenylephrine. Meloxicam (0.4 mg/kg intramuscularly) and buprenorphine (10 µg/kg intramuscularly) were used for postoperative analgesia.

Postoperative care

Each day, the health of the animals was checked and diuretics were administered orally (furosemide 60 mg and spironolactone 100 mg). The central venous pressure, oxygen saturation, weight and blood values were documented weekly. Paracentesis or thoracentesis was performed when ascites or pleural effusions were clinically diagnosed. The animals were sacrificed after 21 weeks or after development of end-stage failure of the Fontan physiology. End-stage Fontan failure was defined as severe clinical deterioration or the development of therapy-refractory pleural effusions or ascites. The weight and condition of the animals were documented before the animal was euthanized by an intravenous injection of pentobarbital sodium (150 mg/kg Euthasol 40%, Virbac USA, Fort Worth, TX, USA). A postmortem examination was performed with inspection of pleural spaces and the abdominal cavity for ascites or pleural effusions and the anastomoses for the presence of thrombus or stenosis.

Haemodynamics

Heart rate, arterial blood pressure, central venous pressure and CO were recorded at the beginning of the procedure and after Fontan completion. Arterial and mixed venous blood samples were taken at the start and at the end of the procedure and analysed using the Epop blood gas and electrolyte analysis system (Alere, Waltham, MA, USA). These parameters and samples were only recorded when the animal was stable, with a closed thoracic cavity and free from haemodynamic support. CO was determined by the Fick principle:

$$CO = VO_2 / (C_a - C_v).$$

The oxygen concentration of arterial blood (C_a) and the oxygen concentration of mixed venous blood (C_v) were estimated by using the following equations:

$$C_a = Hb \times 1.34 \times O_2sat_a + 0.0032 \times PO_{2a}$$

and

$$C_v = Hb \times 1.34 \times O_2sat_v + 0.0032 \times PO_{2v},$$

where Hb is the blood content of haemoglobin; O_2sat_a and O_2sat_v are the arterial and mixed venous oxygen saturation, respectively; and PO_{2a} and PO_{2v} are the partial pressures of oxygen in arterial and mixed venous blood, respectively.

The oxygen consumption (VO_2) was estimated by the difference between the oxygen amount in inspired and expired air:

$$VO_2 = (V_I \times \%O_{2I}) - (V_E \times \%O_{2E}),$$

where V_I is the measured inspired volume, $\%O_{2I}$ is the oxygen percentage measured in inspired air and $\%O_{2E}$ is the oxygen percentage measured in expired air. Because the nitrogen amount is

a constant in inspired and expired air, the volume of expired air (V_E) can be estimated using the Haldane transformation:

$$V_E = \left(V_I \times \frac{\%N_{2I}}{\%N_{2E}} \right),$$

where $\%N_{2I}$ and $\%N_{2E}$ are the percentages of nitrogen in inspired and expired air. The oxygen consumption was then calculated by integrating the Haldane transformation in the VO_2 equation:

$$VO_2 = V_I \left(\%O_{2I} - \left(\frac{\%N_{2I}}{\%N_{2E}} \times \%O_{2I} \right) \right).$$

The nitrogen concentrations in inspired ($\%N_{2I}$) and expired ($\%N_{2E}$) air were calculated using the measured concentrations of oxygen, carbon dioxide and isoflurane in inspired and expired air:

$$\%N_{2I} = \left(100 - (\%O_{2I} + \%CO_{2I} + \%ISO_{2I}) \right)$$

and

$$\%N_{2E} = \left(100 - (\%O_{2E} + \%CO_{2E} + \%ISO_{2E}) \right),$$

where $\%O_{2I}$ and $\%O_{2E}$ are the percentages of oxygen measured in inspired and expired air, $\%CO_{2I}$ and $\%CO_{2E}$ are the percentages of carbon dioxide measured in inspired and expired air, $\%ISO_I$ and $\%ISO_E$ are the percentages of isoflurane measured in inspired and expired air.

Preoperative and postoperative exercise testing

The speed was set at 5.5 km/h and the inclination was increased by 2% every 2 min until a maximum inclination of 10% was reached at 10 min. The exercise continued until exhaustion or after 14 min. Heart rate was recorded every 2 min.

Magnetic resonance imaging

A subgroup ($n=6$) of animals were considered stable enough for general anaesthesia. A magnetic resonance imaging (MRI) scan was performed under general anaesthesia on this small subgroup of animals. Two healthy animals underwent MRI scans to serve as controls. Animals were scanned in left lateral decubitus with a 3.0-Tesla MRI (Magnetom, Trio Tim; Siemens Medical Solutions, Erlangen, Germany) with a phased-array surface receiver coil wrapped over the heart. Images were acquired with electrocardiographic gating and during suspended respiration. Cine images were acquired in vertical and horizontal long- and short-axis planes. The following parameters were analysed using an in-house developed software program (RightVol, KU Leuven, Belgium): right and left ventricular end-diastolic and end-systolic volume, right and left ventricular stroke volume and left and right ventricular output. LV and RV epicardial and endocardial contours were manually traced on a stack of short-axis image slices with simultaneous reference to the long-axis plane. End-diastolic and end-systolic volumes were calculated by a summation of disks. CO was measured as the product of stroke volume and heart rate.

Data analysis

Data analysis was limited to descriptive statistics and was performed using SPSS Statistics 24 (IBM corp., Armonk, NJ, USA) and Microsoft Office Excel 2016 (Microsoft corp., Redmond, WA, USA). All data are expressed as the median and IQR.

RESULTS

Surgical procedure

All 15 animals survived the procedure and could be weaned from the ventilator within 30 min. Average surgical time and superior vena cava–right atrium bypass time were 245 (IQR 209–293) and 25 (IQR 19–27) min, respectively.

Haemodynamic characteristics

The average central venous pressure was lower at baseline than after establishment of the TCPC [4 (IQR 3–6) vs 16 (IQR 14–17) mmHg]. TCPC induced a decrease in mean arterial blood pressure from 68 (IQR 54–75) to 52 (IQR 50–61) mmHg. CO decreased from 5.1 (IQR 4.6–6.8) to 1.7 (IQR 1.3–2.7) l/min. There was no observed difference in heart rate [83 (IQR 76–92) vs 82 (IQR 80–89) bpm] or oxygen saturation [100 (IQR 99–100)% vs 100 (IQR 98–100)%].

Survival

Three animals recovered very slowly after surgery and died suddenly within 3 weeks postoperatively. Three animals died during induction of general anaesthesia for MRI, reflecting their poor general condition. Four animals suffered from progressive failure of the Fontan physiology with therapy-refractory pleural effusions and were sacrificed after 24, 89, 101 and 128 days. In all cases, postmortem examination revealed no signs of thrombus or obstruction of the TCPC.

Exercise test

Only 4 animals of the subgroup of animals that underwent a preoperative exercise test ($n=8$) were judged to have sufficiently recovered after 3 weeks to perform a postoperative exercise test. Preoperatively, a slow increase in the heart rate was observed until a maximum of 230 (IQR 225–240) bpm was reached at the end of the exercise test ($T=14$ min). All animals with a TCPC stopped the test because of exhaustion. The test was stopped after a median time of 6.6 (IQR 4.4–8.3) min and in the animal(s) with a TCPC, a maximum heart rate of 197 (IQR 160–200) bpm was reached.

Magnetic resonance imaging

Only a subgroup ($n=6$) of animal(s) with a TCPC were considered stable enough to undergo general anaesthesia for MRI. MRI was unsuccessful in 3 of these animals because they died during induction of general anaesthesia. The data of the MRI analysis are summarized in Table 1. There is an observed reduction in the right ventricular end-diastolic volume, stroke volume and output in animals with a TCPC ($n=3$) compared to healthy controls

Table 1: MRI characteristics

	Healthy control ($n=2$), median (IQR)	TCPC ($n=3$), median (IQR)
Left ventricle		
End-diastolic volume (ml)	85 (73–98)	88 (62–88)
End-systolic volume (ml)	51 (41–60)	39 (28–41)
Stroke volume (ml)	35 (32–37)	47 (33–49)
Ventricular output (l/min)	4.15 (3.77–4.51)	5.28 (3.8–5.79)
Right ventricle		
End-diastolic volume (ml)	50 (44–56)	20 (16–21)
End-systolic volume (ml)	17 (14–20)	15 (12–17)
Stroke volume (ml)	33 (30–36)	2 (2–4)
Ventricular output (l/min)	3.94 (3.53–4.35)	0.22 (0.20–0.42)

IQR: interquartile range; MRI: magnetic resonance imaging; TCPC: total cavopulmonary connection.

($n=2$). Figure 4 illustrates the difference in right ventricular end-diastolic volume between a TCPC animal and a healthy control.

DISCUSSION

Over the last decades, there has been a gradual improvement in outcome for patients with a functionally univentricular heart, thanks to steady advancements in the available treatment options [2]. These advancements have been supported by an increasing comprehension of the Fontan physiology and often guided by *in vivo* and *in vitro* experiments.

In 1966, Haller *et al.* [13] established the first complete bypass of the RV in an animal model through a 2-staged right atrium–pulmonary artery connection. Following the introduction of the Fontan palliation in clinical practice, other experimental right atrium–pulmonary artery models attempted to characterize the Fontan circulation [14–16, 23]. These studies showed that right atrial contractions were not needed to maintain pulmonary flow [15] and that it was important to protect the coronary venous system from excessive pressure [16]. These and other insights eventually led to the exclusion of the right atrium from the Fontan circulation and the introduction of the TCPC [24]. The TCPC results in improved haemodynamic efficiency and reduces the incidence of right atrium-related complications [2]. Because of these improvements to the Fontan palliation, there is an increasing population of patients living with a Fontan physiology. Despite these recent surgical advances, Fontan patients still suffer from a heavy burden of disease and it has been estimated that the need for advanced heart failure such as cardiac transplantation will only increase in the next decades [25]. In the future, mechanical assist devices that can re-establish a biventricular physiology might play an important role in the management of failing Fontan patients and serve as bridge-to-recovery, bridge-to-transplant or destination therapy. Currently, there are no Fontan assist devices clinically available despite an increasing preclinical interest [7–12]. Therefore, there is an increasing clinical need for a chronic failing TCPC animal model that can investigate the efficacy of Fontan assist devices and different mechanical support strategies.

Over the last 30 years, several experimental studies have established modified TCPCs in animal models, with or without the use of cardiopulmonary bypass [17–22]. However, to this day, it has

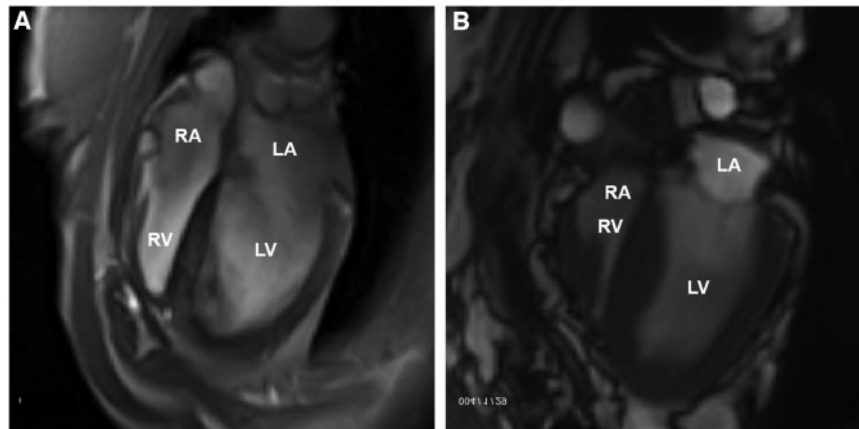


Figure 4: Long-axis magnetic resonance imaging from a normal control (**A**) and a chronic Fontan animal (**B**). Note the significantly smaller right ventricular cavity in animals with a Fontan circulation. LA: left atrium; LV: left ventricle; RA: right atrium; RV: right ventricle.

proved extremely demanding to develop a chronic animal model of the TCPC, thereby limiting these studies to acute experiments or models of an incomplete bypass of the right heart. In the aforementioned experimental studies, the TCPC is established using a Y-shaped construct, using either a graft or cannulas, to connect the superior and inferior caval veins to the main pulmonary artery or to the right pulmonary artery.

For our study, the superior vena cava was anastomosed end-to-side to the pulmonary artery and the inferior vena cava was connected separately to the pulmonary artery by an extracardiac conduit. The connection between the inferior vena cava and the right atrium was ligated to establish a complete diversion of the systemic venous blood to the pulmonary artery. Connecting both caval veins separately to the pulmonary artery was technically challenging but was imperative in ensuring adequate blood flow to the pulmonary artery. Cavopulmonary flow depends on the vascular resistance and the vascular resistance is very sensitive to changes in the radius of the vessels. Therefore, the size of the anastomotic area between the pulmonary artery and both the superior vena cava and the conduit is considered critical to maintain an adequate volume of blood flow from the systemic veins to the pulmonary artery. This modification to the surgical technique could potentially explain the haemodynamic stability we encountered after Fontan completion and the relative successful postoperative period and survival of the animals.

The haemodynamic changes that were observed after completion of the TCPC were in accordance with previous studies that observed a significant increase in central venous pressure and a significant decrease in mean arterial pressure after Fontan completion [21–23]. However, compared to the data shown by these investigators, the decrease in mean arterial pressure in our study appears to be less severe. The heart rate appeared to remain stable in accordance with the data of Szabó *et al.* and Wang *et al.* [20, 22]. This is in contrast with the data from some experimental protocols that showed a significant decrease in heart rate [17, 19, 21, 23]. There was an important decrease in CO, as in previous studies [17, 19–21, 23].

Before this study, we performed a pilot study that allowed us to gain experience in the perioperative management and to become proficient in performing this technically challenging procedure. We learned that, following Fontan completion, the animals suffer from low output syndrome and develop severe lactic acidosis, leading to a high mortality during the first hours

postoperatively. Therefore, all animals were under observation for the first 6–12 h postoperatively and an arterial blood gas sample was taken every 30–60 min. Metabolic abnormalities were closely monitored and corrected accordingly; in some cases, oxygen was administered via a face mask. Once the lactate level had dropped and the metabolic state was normalized, the animals were placed in the stable. The animals in our pilot study also developed significant pleural effusions and ascites. Therefore, all animals were placed on diuretics and, if indicated, thoracentesis or paracentesis was performed. Our perioperative management, with intensive supportive therapy during the first 12 h postoperatively, probably explains the relative success we had in surviving the animals. Despite all these interventions and a very close follow-up, the animals remained in a very precarious condition. This was also demonstrated by the high mortality (50%) during induction of general anaesthesia for MRI.

MRI showed that left ventricular volumes and function were preserved. The RV was characterized by a significant decrease in end-diastolic diameter, stroke volume and ventricular output. Because blood from the coronary sinus and the Thebesian veins was still flowing in the RV, the pulmonary artery was not ligated. This allowed for decompression of the RV. Therefore, there was still a limited right ventricular output.

To characterize the failing Fontan physiology of our animals, we attempted to demonstrate their functional capacity by an exercise test. Before the procedure, the animals could complete the entire exercise protocol, while all Fontan animals stopped the exercise prematurely because of exhaustion. The maximum heart rate reached was lower after the Fontan procedure. This would be in accordance with the clinical findings of Durongpisitkul *et al.* [26], who reported a slightly reduced maximum heart rate in patients with a Fontan circulation compared to healthy individuals.

Limitations

Naturally, there are several limitations in the design of our model. We established the TCPC in healthy, adult animals, which is a fundamentally different situation from the paediatric univentricular physiology. These patients develop Fontan failure over the course of decades, whereas in our animal model failure of the Fontan physiology developed over several weeks. Additionally, the

Fontan population is a heterogeneous group of patients supported by either an anatomic RV or LV, while in our animal model all animals have an anatomic systemic LV. Furthermore, while in the clinical setting the Fontan circulation is established in 3 stages, we have chosen to perform the procedure in 1 stage. In our study, the caval veins were connected to the main pulmonary artery, while in the clinical setting the caval veins are connected to the right pulmonary artery. Although all caval blood was diverted to the pulmonary artery, the blood from the coronary sinus and the Thebesian veins continued to be ejected by the RV in the pulmonary artery to allow for decompression of the RV.

The mortality was high despite these technical modifications and close follow-up, illustrating the severity of the assault imposed on the normal physiology by an acute elimination of the RV. The data on exercise capacity and MRI are limited to a small subgroup of animals and cut down because of the high mortality. Despite these limitations, this study demonstrates the feasibility of a chronic animal model of unsupported Fontan physiology, with 5 animals surviving for 21 weeks.

CONCLUSION

In conclusion, this study demonstrates that it is feasible to create a chronic animal TCPC model of unsupported Fontan physiology. Our data confirmed the distinctly abnormal haemodynamics associated with an acute TCPC. Moreover, the Fontan circulation in our study provided all hallmarks of a failing Fontan physiology (chronic venous congestion, impaired exercise capacity, ascites and pleural effusions) and was associated with a significant risk of progressive failure and death. In the future, this experimental model could facilitate studies on the pathophysiology of the failing Fontan circulation and might play a crucial role in the development of advanced approaches, such as cavopulmonary assist devices, to treat patients with a failing Fontan circulation.

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Conflict of interest: none declared.

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